

Patent Claims:

1. A method for identifying an agent that modulates the activity of an enzyme comprising
- a) providing a phosphate catalyzing enzyme,
 - 5 b) providing a peptide,
 - c) optionally providing a phosphate containing donor,
 - d) providing a candidate compound,
 - e) contacting the enzyme of step a) with a peptide of step b) and optionally the phosphate
10 containing donor of step c) in the absence of d) and in the presence of d) for a
predetermined period of time so that an enzymatic product can be formed,
 - f) transferring at least an aliquot of the enzymatic product formed in step e) to a solid phase
which is capable to bind to the peptide in the enzymatic product,
 - g) detecting the enzymatic product bound to the solid phase,
 - h) determining whether there is a difference in the amount of enzymatic product in the
15 absence and in the presence of a candidate compound in step e), and
 - i) choosing an agent that modulates the activity of an enzyme, which method is
characterized in that
 - I) the enzyme is a PAK kinase,
 - the peptide comprises the amino acid sequence S-S-L-R-A-S-T,
 - 20 - the phosphate containing donor of step c) is present in step e), and
 - the enzymatic product detected in step g) is the amount of phosphoserine and/or
phosphothreonine in the bound peptide; OR
 - II) the enzyme is CD45 tyrosine phosphatase,
 - the peptide comprises the amino acid sequence R-N-Q-E-T-Y-E-T-L-K-H or
25 A-E-N-T-I-T-Y-S-L-L-M-H-P, wherein Y is phosphorylated tyrosine,
 - the phosphate containing donor of step c) is absent in step e), and
 - the enzymatic product detected in step g) is the amount of phosphotyrosine in the bound
peptide.
- 30 2. A method according to claim 1 wherein the amount of phosphoserine and/or
phosphothreonine is determined with an antibody selected from the group consisting of
phosphoserine recognizing antibody, phosphothreonine recognizing antibody and
phosphoserine and phosphothreonine recognizing antibody and the amount of
phosphotyrosine is determined with a phosphotyrosine recognizing antibody.

3. A method according to any one of claims 1 or 2 wherein the enzyme is a PAK kinase and peptide has the amino acid sequence A-K-R-R-R-L-S-S-L-R-A-S-T-S-K-S.
- 5 4. A kit for identifying an agent that modulates the activity of a PAK kinase comprising as components
- a) a PAK kinase,
 - b) a peptide comprising the amino acid sequence S-S-L-R-A-S-T,
 - c) a phosphate containing donor,
 - 10 d) means for detecting phosphoserine and/or phosphothreonine, and
 - e) optionally a solid phase.
5. A kit for identifying an agent that modulates the activity of CD45 tyrosine phosphatase comprising as components
- 15 a) a CD45 tyrosine phosphatase,
 - b) a peptide comprising the amino acid sequence R-N-Q-E-T-Y-E-T-L-K-H or A-E-N-T-I-T-Y-S-L-L-M-H-P, wherein Y is phosphorylated tyrosine,
 - c) means for detecting phosphotyrosine, and
 - d) optionally a solid phase.
- 20 6. A method for differentiating whether an agent is capable to modulate the serine specific activity of a PAK kinase or the threonine specific activity of a PAK kinase or both comprising
- a) providing a PAK kinase,
 - 25 b) providing a peptide comprising the amino acid sequence S-S-L-R-A-S-T,
 - c) providing a phosphate containing donor,
 - d) providing a candidate compound,
 - e) contacting the enzyme of step a) with a peptide of step b) and the phosphate containing donor of step c) in the absence of d) and in the presence of d) for a predetermined period
 - 30 of time so that a phosphoserine and/or phosphothreonine containing peptide can be formed,
 - f) transferring at least an aliquot of the peptide formed in step e) to a solid phase which is capable to bind to the peptide,
 - g) detecting the amount of phosphoserine and phosphothreonine in the bound peptide by

use of at least 2 different antibodies selected from the group consisting of phosphoserine recognizing antibody, phosphothreonine recognizing antibody and phosphoserine and phosphothreonine recognizing antibody, and

h) determining if the agent modulates the serine specific activity of a PAK kinase, or the
5 threonine specific activity of a PAK kinase, respectively, or both.

7. A method according to claim 6 wherein the antibodies bear a different label and the amount of phosphoserine and phosphothreonine is determined simultaneously.

10 8. The use of a peptide comprising the amino acid sequence S-S-L-R-A-S-T for identifying an agent that modulates the activity of a PAK kinase.

9. The use of a peptide comprising the amino acid sequence S-S-L-R-A-S-T for
differentiating between an agent that is capable to modulate the serine specific activity of
15 a PAK kinase or the threonine specific activity of a PAK kinase or both.